

Claims:

1. A prokaryotic cell that is genetically modified to shift the redox status of the cytoplasm to a more oxidative state, and which further contains a gene encoding a catalyst of disulfide bond formation and/or isomerization.
- 5 2. The prokaryotic cell of claim 1, wherein the expression or activity of a reductase is decreased relative to that in the corresponding wild type cell.
3. The prokaryotic cell of claim 2, wherein the reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase, and glutathione.
4. The prokaryotic cell of claim 3, in which the expression or activity of a second
10 reductase is decreased relative to that in the corresponding wild type cell.
5. The prokaryotic cell of claim 4, wherein the second reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase, and glutathione.
6. The prokaryotic cell of claim 2, wherein the gene encoding the reductase is mutated.
7. The prokaryotic cell of claim 6, wherein the gene encoding the reductase contains a
15 null mutation.
8. The prokaryotic cell of claim 5, wherein the genes encoding the first and the second reductases contain a null mutation.
9. The prokaryotic cell of claim 2, wherein the activity of the reductase is inhibited.
10. The prokaryotic cell of claim 9, wherein the activity of the reductase is inhibited by
20 contacting the prokaryotic cell with an agent.
11. The prokaryotic cell of claim 1, further modified to increase its ability to proliferate.
12. The prokaryotic cell of claim 4, further modified to increase its ability to proliferate.
13. The prokaryotic cell of claim 11, wherein the modification consists of the introduction of a suppressor mutation.
- 25 14. The prokaryotic cell of claim 12, wherein the modification consists of the introduction of a suppressor mutation.
15. The prokaryotic cell of claim 11, wherein the modification restores at least some of the reducing capacity to the cytoplasm of the prokaryotic cell.
16. The prokaryotic cell of claim 11, wherein the modification is a mutation in the *ahpC*
30 gene which reduces its peroxidase activity.
17. The prokaryotic cell of claim 16, wherein the mutation is located in a region containing four triplet repeats.
18. The prokaryotic cell of claim 17, wherein the mutated *ahpC* protein has the amino acid sequence set forth in SEQ ID NO: 24.
- 35 19. The prokaryotic cell of claim 1, having ATCC Designation No. PTA-938 (FA112).

20. The prokaryotic cell of claim 1, having ATCC Designation No. PTA-939 (FA113).
21. The prokaryotic cell of claim 19, further comprising a nucleic acid encoding a catalyst of disulfide bond formation or isomerization.
22. The prokaryotic cell of claim 20, further comprising a nucleic acid encoding a catalyst of disulfide bond formation or isomerization.
23. A prokaryotic cell of claim 1, further comprising a heterologous nucleic acid.
24. The prokaryotic cell of claim 1, which comprises a nucleic acid encoding a catalyst of disulfide bond isomerization.
25. The prokaryotic cell of claim 24, which comprises a nucleic acid encoding a catalyst of disulfide bond isomerization.
- ~~26. The prokaryotic cell of claim 25, wherein the catalyst is a DsbC protein or an analog thereof.~~
27. The prokaryotic cell of claim 1, wherein the catalyst is a variant of a protein of the thioredoxin superfamily having a redox potential that is higher than that of its wild type counterpart.
28. The prokaryotic cell of claim 27, wherein the variant is a "Grx" variant of thioredoxin A.
- ~~29. A prokaryotic cell that is genetically modified to shift the redox status of the cytoplasm to a more oxidative, and which further contains a genetic modification to increase its ability to proliferate.~~
- ~~30. The prokaryotic cell of claim 29, in which the expression or activity of a reductase is decreased relative to that in the corresponding wild type cell.~~
- ~~31. The prokaryotic cell of claim 30, wherein the reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase, and glutathione.~~
- ~~32. The prokaryotic cell of claim 30, in which the expression or activity of a second reductase is decreased relative to that in the corresponding wild type cell.~~
- ~~33. The prokaryotic cell of claim 29, wherein the second reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase, and glutathione.~~
34. The prokaryotic cell of claim 30, wherein the gene encoding the reductase is mutated.
35. The prokaryotic cell of claim 34, wherein the gene encoding the reductase contains a null mutation.
36. The prokaryotic cell of claim 32, wherein the genes encoding the first and the second reductases contain a null mutation.
37. The prokaryotic cell of claim 30, wherein the activity of the reductase is inhibited.
38. The prokaryotic cell of claim 37, wherein the activity of the reductase is inhibited by contacting the prokaryotic cell with an agent.

39. The prokaryotic cell of claim 29, wherein the genetic modification is a suppressor mutation.

40. The prokaryotic cell of claim 29, wherein the modification restores at least some of the reducing capacity to the cytoplasm of the prokaryotic cell.

5 41. The prokaryotic cell of claim 40, wherein the modification is a mutation in the *ahpC* gene which reduces its peroxidase activity.

42. The prokaryotic cell of claim 41, wherein the mutation is located in a region containing four triplet repeats.

10 43. The prokaryotic cell of claim 42, wherein the mutated *ahpC* protein has the amino acid sequence set forth in SEQ ID NO: 24.

44. The prokaryotic cell of claim 29, further containing a gene encoding a catalyst of disulfide bond formation and/or isomerization.

20 45. ~~The prokaryotic cell of claim 44, wherein the catalyst is a DsbC protein.~~

15 46. The prokaryotic cell of claim 44, wherein the catalyst is a variant of a protein of the thioredoxin superfamily having a redox potential that is higher than that of its wild type counterpart.

47. The prokaryotic cell of claim 46, wherein the variant is a "Grx" variant of thioredoxin A.

20 48. The prokaryotic cell of claim 44, wherein expression of the gene encoding the catalyst is inducible.

49. A method for producing a protein having at least one disulfide bond, comprising growing a host cell of claim 1 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein from the host cell.

25 50. A method for producing a protein having at least one disulfide bond, growing a host cell of claim 29 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein from the host cell.

51. A protein produced by the method of claim 49.

30 52. A protein produced by the method of claim 50.

53. Tissue plasminogen activator (tPA) produced by the method of claim 49.

54. Tissue plasminogen activator (tPA) produced by the method of claim 50.

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